Search for alternate hosts of the coconut Cape Saint Paul Wilt Disease pathogen

Egya Ndede YANKEY[1]
Fabian PILET[2]
Robert Nketsia QUAICOE[1]
Sylvester Kuuna DERY[1]
Michel DOLLET[2]
Victoria Pearl DZOGBEFIA[3]

[1] *CSIR-OPRI, coconut Research Programme, Box 245 Sekondi, Ghana*
<ndedeyankey@yahoo.com>
[2] *CIRAD, Research Unit 29, Etiology/Wilts, Dept. Biological systems*
[3] *KNUST, Faculty of Biosciences, Kumasi, Ghana*

Abstract: *Lethal Yellowing disease locally called Cape Saint Paul wilt disease (CSPWD) is the bane of the coconut industry in Ghana and is caused by a phytoplasma. In Ghana, there are areas where the disease has re-infected re-plantings long after decimating all the palms in the area. This brings to the fore the possibility of alternate hosts in the spread of the disease because the pathogen is an obligate parasite. In this work, a number of plants were screened for their host status to the CSPWD pathogen. The presence of phytoplasmas in these plants was tested by polymerase chain reaction analysis using universal phytoplasma primers P1/P7 and CSPWD-specific primers G813/GAKSR. Although* Desmodium adscendens *tested positive to the CSPWD-specific primers, cloning and sequencing did not confirm it as an alternate host. The identification of alternate hosts will help us to evolve sound control strategies against the spread of the disease.*

Key words: *Ghana, coconut, phytoplasmas, alternate hosts*

The coconut palm (*Cocos nucifera* L.) is considered the most important crop along the coastal belt of West Africa and can be grown (with minimal capital outlay) in poor sandy salt-loaded soils where very few or no other crop would survive [1, 2]. It is reported that about 4.2% of Ghana's population depend on coconut for their livelihood [3]. The cultivation, processing and marketing of the crop supports the livelihoods of many poor coastal people and help sustain the environment [4]. In 1932, a disease called Lethal Yellowing (LY) was detected among some palms in the Volta Region of Ghana and ever since the fortunes of the coconut industry has been on the decline.

LY is a highly destructive and fast spreading disease affecting coconut palm and at least 35 other palm species in the Americas [5]. The disease has brought great distress to several rural coastal communities engaged in the coconut industry in Ghana, leaving them without a sustainable source of livelihood [6]. The destruction of the coconut palms has environmental repercussions too as previously covered lands become exposed leaving these areas prone to degradation.

LY diseases are caused by phytoplasmas, which are essentially cell wall-less prokaryotes belonging to the class Mollicutes. Plant-to-plant transmission of these pathogens are carried out by insect vectors (plant hoppers and leaf hoppers), through vegetative propagation of infected plant materials or by graft inoculation [7]. Phytoplasmas cannot be cultured *in vitro*, a phenomenon due probably to the lack of essential genes and functions [8].

After the occurrence of LY diseases in 1964 at Cape Three Points in the Western Region (WR) of Ghana, in 1977, the Crops Research Institute of the Council for Scientific and Industrial Research (CSIR-CRI) planted in some varieties to evaluate their resistance to the disease. All the varieties succumbed to the disease [6]. Then in 1981, the Ministry of Agriculture under the France-Ghana-Cote d'Ivoire Coconut Project brought in some varieties from outside the country to screen for their resistance to the disease. The disease decimated these palms as

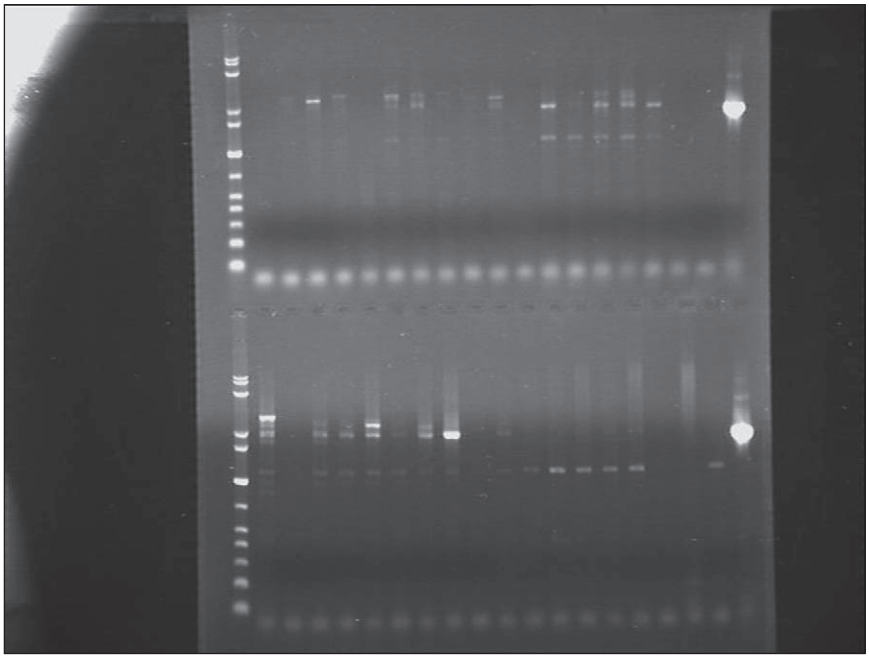

Figure 1. *Amplification of samples with primers P1/P7.*

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well with the exception of the Sri Lanka Green Dwarf (SGD) variety [6]. In 1995, the CSIR – Oil Palm Research Institute, Coconut Research Programme under the European Commission of Science and Technology for Development III Project also imported some varieties from Cote d'Ivoire for resistance trials. Yet again, the disease has cleared almost all the palms (62.3%) [9]. It is in the light of this history that the possibility of the presence of alternate hosts of the Cape Saint Paul Wilt Disease (CSPWD) phytoplasma was investigated. The hypothesis being that the phytoplasma, an obligate parasite, needed another host to survive during the periods when their primary hosts, the coconuts, were unavailable due to death from the disease. In the Americas, LY phytoplasma is known to have other hosts other than the coconut. This situation may hold true for other areas affected by similar LY-type coconut diseases, such as CSPWD.

In choosing the species to sample, priority was given to plants related to known hosts of the coconut LY phytoplasma. *Emilia fosbergii* and *Synedrella nodiflora* are reported to be alternate hosts in Jamaica [10], so *Emelia sonchifolia* and *S. nodiflora* were sampled. Plants that have been reported to host any type of phytoplasma were also sampled. As a result, pepper, tomato, potato, cassava, sugar cane, *Solanum* spp. and *Euphorbia* spp., which are reported to host phytoplasmas [11-14] were sampled. Plants showing any of the general symptoms of phytoplasma infection such as stunting, yellowing, withering, witches' broom (proliferating shoots) [15] as well as some asymptomatic plants were also selected.

Materials and methods

Sampling sites

Sampling of plant species was carried out from two locations: Cape Three Points in the WR and Asebu in the Central Region (CR). Cape Three Points is approximately 66 km southwest from Sekondi. Asebu is approximately 100 km northeast from Sekondi; both are active disease foci.

Sampling period

Sampling was done twice in a year: one in the rainy season and the other in the dry season. This was done to take account of the effect of changes in environmental conditions on the dynamics of plants and insect life. For example, some plants that cannot withstand drought die out during the dry season and this may be true for the insect vector population fluctuation as well. The implication is that some potential hosts of the pathogen can be missed if sampling is not done in the two seasons. The rainy season sampling took place from July to December 2007 and that of the dry season was done from March to April 2008.

Sampling methodology

The sampled species were mainly grasses, a few shrubs and a few tree plants. Fifteen species were sampled per location in each of the seasons. A total of 57 species were sampled during the period (see *tables 1 and 2* for the lists of plant species sampled for analysis). The plants were sampled within and around the two trial fields as well as from some coconut farms surrounding the trial plots. For every species, 30 individual plants were picked. A few, however, due to their rarity did not meet this number.

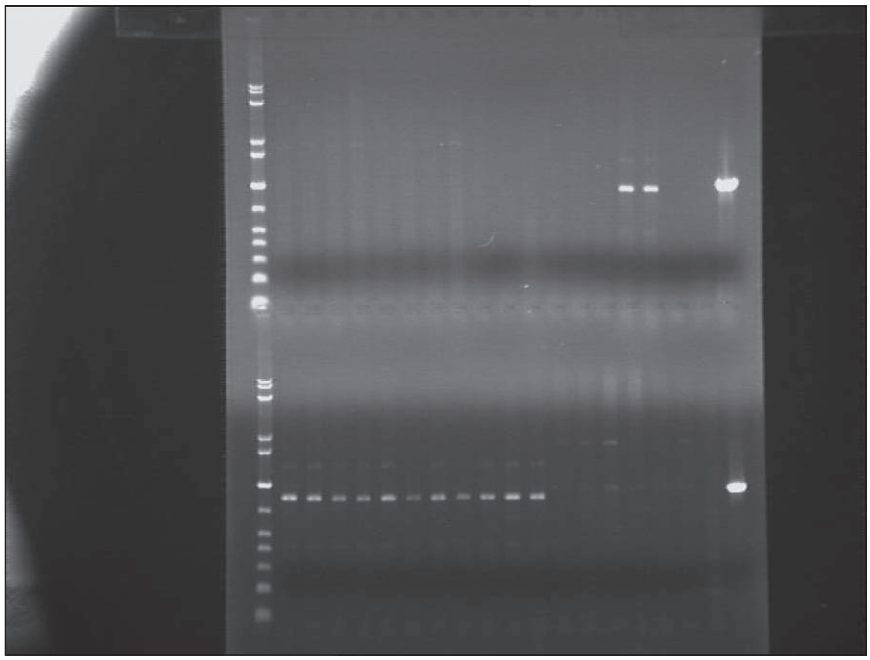

Figure 2. *Amplification of* Desmodium adscendens *samples with G813/GAKSR.*

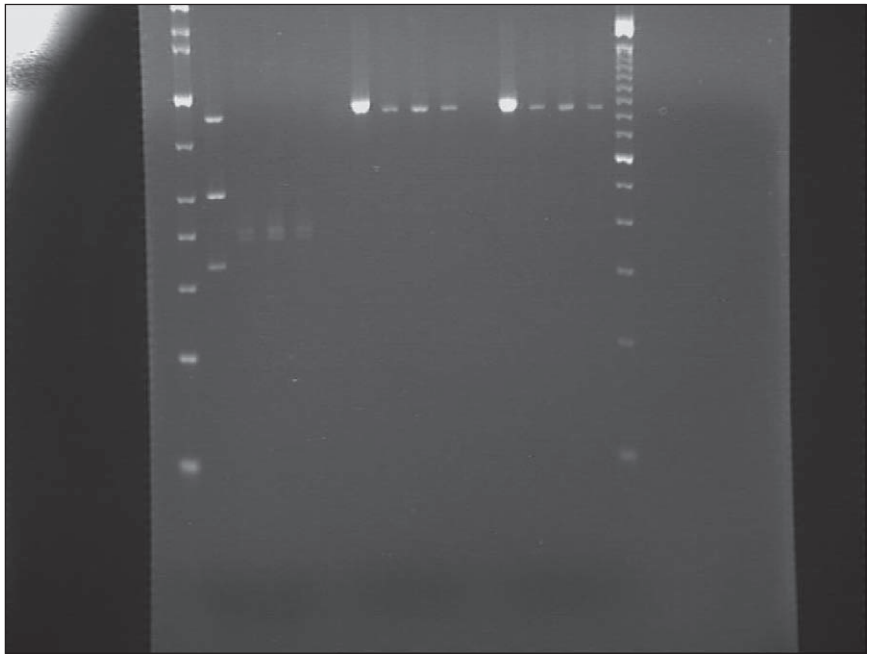

Figure 3. *Restriction fragment length polymorphism (RFLP) analysis of three samples of* Desmodium adscendens *(G813/GAKSR products) using three restriction endonucleases.*

Total DNA extraction from plant tissue

For each species, two individual plants were pooled together to give one sample. Hence, 15 DNA samples were prepared for each species. One gram of each sample was ground in 5-mL Cetyl trimethyl ammonium bromide (CTAB) and the DNA extracted according to the protocol of Daire *et al.*, 1997 [16].

Polymerase chain reaction and gel electrophoresis

The DNA samples were assayed for the presence of phytoplasmas using phytoplasma universal primer pair P1 (5′-AAG AGT TTG ATC CTG GCT CAG GAT T-3′) [17] and P7 (5′-CGT CCT TCA TCG GCT CTT-3′) [18]. Positives from this test were screened for the CSPWD phytoplasma using primers G813f (5′-CTA AGT GTC GGG GGT TTC C-3′) and GAKSR (5′-TTG AAT AAG AGG AAT GTG G-3′) [19]. The polymerase chain reaction (PCR) was performed using a PTC-100 Programmable Thermal Controller manufactured by MJ Research Inc (Waltham, Massachusett, USA). The PCR products were analysed and visualised by gel electrophoresis on a 0.8% agarose gel prepared using Tris-Borate-Ethylenediaminetetraacetic acid (EDTA) buffer (TBE) (1×) and stained with ethidium bromide. A 1-kb ladder (Invitrogen/Quiagen) was loaded in either the first or last lane. The gel was visualised and photographed under UV light. Negative controls consisted of reaction mixtures with DNA template from healthy coconut palms. DNA templates from CSPWD-infected palms were used for the positive controls and the blanks had the DNA templates substituted with water.

Table 1. *Results of assay of rainy season samples.*

	Primers					
	P1/P7			**G813/GAKSR**		
	No. of samples	**No. of positives**	**% +**	**No. of samples**	**No. of positives**	**%+ve**
Plant species (Asebu)						
Commelina benghalensis	15	7	46.7	7	0	0
Solanum torvum	15	0	0	0	0	0
Calapogonium mucunoides	15	2	13.3	2	0	0
Desmodium adscendeus	15	13	86.7	13	13	100
Malacantha alnifolia	15	4	26.7	4	0	0
Panicum maximum	15	1	6.7	1	0	0
Citrus limon	15	3	20	3	0	0
Nephrolepis biserrata	15	0	0	0	0	0
Sida acuta	15	0	0	0	0	0
Tridax procumbens	15	0	0	0	0	0
Pennisetum purpureum	15	0	0	0	0	0
Ficus exasperata	15	0	0	0	0	0
Oxytenanthera abyssinica	15	0	0	0	0	0
Chromolaena odorata	15	0	0	0	0	0
Rauvolfia vomitoria	15	0	0	0	0	0
Plant species (Cape Three Points)						
Setaria megaphylla	15	0	0	0	0	0
Mimosa pudica	15	0	0	0	0	0
Stachytarpheta indica	15	0	0	0	0	0
Aspilia africana	15	0	0	0	0	0
Borreria scabra	15	0	0	0	0	0
Saccharum officinale	15	0	0	0	0	0
Dissotis rotundifolia	15	0	0	0	0	0
Asystasia gangetica	15	0	0	0	0	0
Ananas sativa	15	0	0	0	0	0
Rauvolfia vomitoria	15	0	0	0	0	0
Flagellaria guineensis	15	0	0	0	0	0
Pueraria phaseoloides	15	0	0	0	0	0
Manihot esculenta	15	0	0	0	0	0
Voacanga africana	15	0	0	0	0	0

Restriction fragment length polymorphism (RFLP)

Samples that tested positive to the CSPWD-specific primers were digested with restriction endonucleases. The enzymes used were RSA1, EcoR1, HindIII and Alu1. The reaction was incubated in a water bath at 37 °C for 9 h. Analysis of digestion product was done by electrophoresis on a 2% agarose gel.

Results

Samples from plants such as *Calapogon mucunoides* (Calapo) and *Desmodium adscendens* were amplified at the expected size of 1.8 kb when primers P1/P7 were applied *(figure 1)*. When the specific primers G813/GAKSR were applied to the positive samples from the P1/P7 test, only the *D. adscendeus* samples were amplified at the expected size of 0.9 kb *(figure 2)*; when restriction digestion was carried out on G813/GAKSR PCR products of *D. adscendeus* samples, RSA 1 did not produce the expected fragment sizes of 498, 311 and 21 kb; however, Hind III and EcoR1 produced fragments corresponding to the profile of the CSPWD phytoplasma of the expected size 855 kb *(figure 3)*. Results of the PCR assay of all the samples are presented in *tables 1 and 2*.

Cloning and sequencing

A sample of *D. adscendeus* was cloned and sequenced by Cogenics society (France). DNA sequences from the sample were determined to be related to *Bacillus megaterium* and *Rhodobacter sphaeroides*.

Discussion

The results so far have failed to identify any alternate hosts of the CSPWD phytoplasma. However, we cannot conclude that there are no alternate hosts in the two areas because only a few plants were screened from the hundreds of plant species that can be found at the two places. Although plants like cassava, sugar cane, *Solanum* spp. and *Stachytarphta* spp. have been reported to host at least a type of phytoplasma elsewhere, this was not the case in this work as no amplifications were obtained from their samples when screened with the phytoplasma universal primer pair P1/P7. This could mean that the phytoplasmas infecting these species are not in the environment of the two sampling sites. The results of this work underscore the need to clone and sequence before making conclusions. This work also demonstrates the need to search for more reliable specific primers for detecting the CSPWD phytoplasma. As far as we are concerned, this work is the first major attempt at finding alternate hosts of the

Table 2. *Results of assay of dry season samples.*

	Primers					
	P1/P7			G813/GAKSR		
	No. of samples	No. of positives	% +	No. of samples	No. of positives	%+ve
Plant species (Asebu)						
Stachytarpheta indica	15	0	0	0	0	0
Desmodium adscendeus	15	0	0	0	0	0
Synedrella nodiflora	10	0	0	0	0	0
Ipomoea involucrata	15	0	0	0	0	0
Lycopersicon esculentum	4	0	0	0	0	0
Jussiae spp.	15	0	0	0	0	0
Sporobolus pyramidalis	15	0	0	0	0	0
Bracharia deflexa	15	0	0	0	0	0
Cymbopogon citrates	15	0	0	0	0	0
Capsicum annuum	15	0	0	0	0	0
Emilia sonchifolia	15	0	0	0	0	0
Eleusine indica	15	0	0	0	0	0
Digitaria adscendens	15	0	0	0	0	0
Euphorbia heterophylla	15	0	0	0	0	0
Solanum tuberosum	15	0	0	0	0	0
Plant species (Cape Three Points)						
Paspalum scrobiculatum	15	0	0	0	0	0
Panicum laxum	15	0	0	0	0	0
Cassytha filiformis	15	0	0	0	0	0
Lantana camara	15	0	0	0	0	0
Clerodendrom capitatum	15	0	0	0	0	0
Morinda lucida	15	0	0	0	0	0
Chassalia kolly	15	0	0	0	0	0
Rottboellia exaltata	15	0	0	0	0	0
Cyperus spp.	15	0	0	0	0	0
Alchornea cordifolia	15	0	0	0	0	0
Sanseviera liberica	15	4	26.7	0	0	0
Pennisetum pedicellatum	15	0	0	0	0	0
Spigelia anthelmia	15	0	0	0	0	0
Phyllanthus amarus	15	0	0	0	0	0
Justica flava	15	0	0	0	0	0

CSPWD phytoplasma and the search needs to continue with more species being screened.

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